

# **Laser Sensor Development for Fluorescence Detection of Plastics and other Anthropogenic Compounds Dissolved in Seawater**

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## **LONG TERM GOALS**

The long-term goal is to build and deploy a highly sensitive, *in situ* AUV-mounted instrument optimized for distinguishing natural colored dissolved organic matter (CDOM) in seawater as well as locating and identifying plastic and rubber-coated objects, such as mines, in the water column and buried within the seafloor. Oceanographic applications include UV-vis spectral characterization of dissolved and particulate (phytoplankton) fluorescence for remote sensing, plume tracking, and pollution applications. The multi-channel UV laser-induced fluorescence sensor (MUVLIFS) will ultimately be integrated and deployed on an autonomous underwater vehicle (AUV) to enhance strategic and oceanographic applications.

## **OBJECTIVES**

The short term goal is to improve the design for a compact UV laser multi-channel fluorometer system for shipboard use in enhanced measurement of UV-stimulated fluorescent compounds in seawater. Currently the compact, dual laser LIF (laser induced fluorescence) system with optical filtered PMT detector provides greatly increased sensitivity by a factor of 80 to 1000 over existing *in situ* instrumentation.

## **APPROACH**

During this funding year (2001-2002) the LIF system was modified to enhance the detection of CDOM in natural waters, including increasing the number of fluorescence emission channels at selected CDOM wavelengths and adding a second laser source. Control electronics were reduced in size for portability. This new laser induced fluorescence (LIF) system was tested on a two day cruise into the

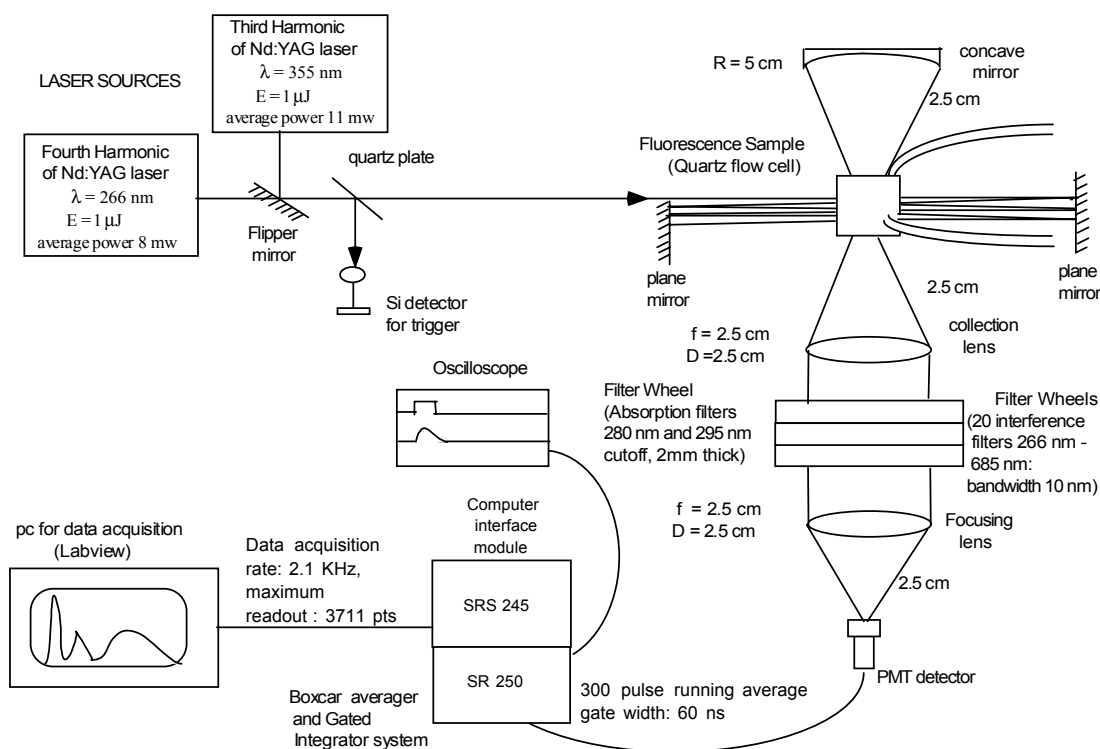
Gulf of Mexico in which a variety of water masses were sampled to determine instrument sensitivity under environmental conditions. This system serves as the prototype for the *in situ* MUVLIFS sensor.

## WORK COMPLETED

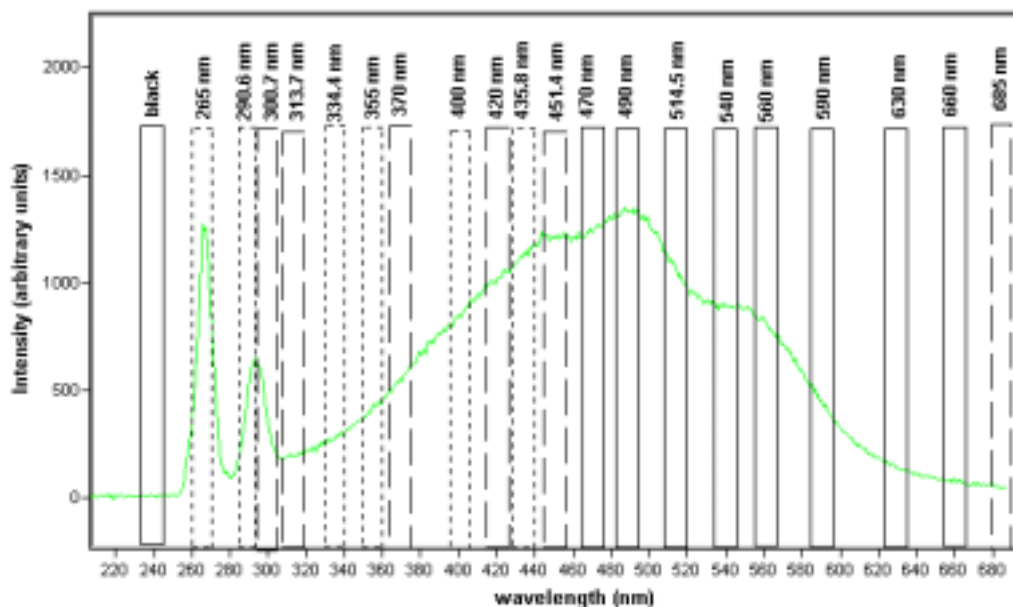
### MUVLIF Development

Further progress was made in the development and testing of the compact multi-channel UV Laser Induced Fluorescence Sensor (MUVLIFS) designed and optimized for detection of trace quantities of anthropogenic substances present in seawater.

During the past year the portable 266 nm LIF system has been outfitted with a second laser source at 355 nm (third Harmonic of Nd:YAG laser). In addition more spectral filters were added so there is now a total of 20 filters to cover the spectral region from 266 nm to 685 nm. A schematic of the portable LIF system is shown in Fig 1. As the figure shows a computer controlled mirror at 45° can be flipped in and out of the beam path in order to allow either the 266 nm laser or the 355 nm laser to be used. Figure 2 shows the positions of the bandwidth filters overlaid on top of the fluorescence spectrum of sea water.



**Figure 1. Schematic of the portable Laser Induced Fluorescence System showing the two laser sources, filter wheels and PMT detector.**

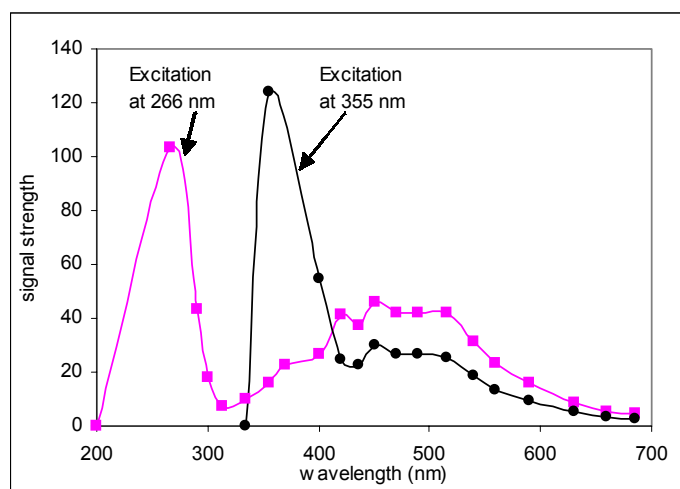


**Figure 2.** Positions of the optical bandpass filters overlaid on top of the fluorescence spectrum of seawater.

## RESULTS

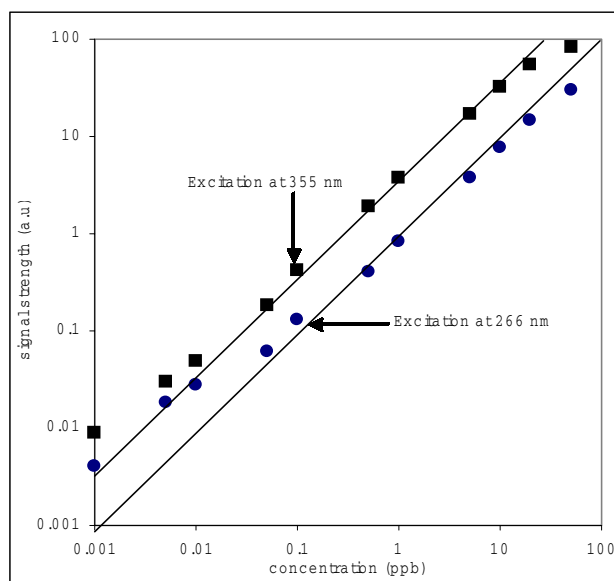
### *MUVLIF Laboratory Measurements*

Initial testing of the system has been conducted, and the fluorescence signal of Bayboro harbor water measured using the modified portable system is plotted in Figure 3. The figure shows the Rayleigh scatter and the fluorescence signal obtained for both excitation lasers. The excitation source at 355 nm will also be used for calibration of the instrument using the transmission at 355 nm.



**Figure 3.** Fluorescence of seawater measured using the LIF system for excitation at 266 nm and 355 nm.

To measure the detection limits and linearity of the fluorescence signal, the fluorescence of quinine sulfate was measured for varying concentrations, ranging from 0.001 ppb to 50 ppb and the results are plotted in Fig. 4. The fluorescence of quinine sulfate showed a linear response for concentrations as low as 0.05 ppb for excitation at 266 nm and about 0.005 ppb for excitation at 355 nm. The detection limit was estimated to be about 0.001 ppb of quinine sulfate for both excitation sources.



**Figure 4. Fluorescence of quinine sulfate at 451 nm for varying concentrations of the sample for excitation at 266 nm and 355 nm. The concentration varied from 0.001 ppb to 50 ppb.**

We plan to extend these studies this next year to continue to improve the MUVLIFS system by automating software and computer control of the system, measuring linearity of the detectors, and reducing the size of the instrument. The system will be used in several extensive field measurements.

#### *MUVLIFS Field Testing*

Performance of the MUVLIFS sensor was evaluated versus that of the existing SAFire and benchtop instruments for sensitivity and selectivity to all compounds of interest in local waters in September 2001. Calibration versus amino acids, quinine sulfate, and dissolved organic carbon (DOC) were performed, as well as full EEM characterization of selected discrete samples. The MUVLIFS system was found to be 80 to 1000 times more sensitive than the commercial instruments. The addition of a second laser for excitation, additional emission bands, and addition of absorption sensors for each excitation will help finalizing this instrument for oceanographic applications.

We have been conducting two other types of investigations to further our understanding of the behavior of artificial and natural CDOM in the ocean under different environmental conditions and enhance our ability to process data from the MUVLIFS at extremely low concentrations of CDOM found in open ocean areas. The first of these are laboratory photodegradation studies, which were conducted during Summer 2002. Our preliminary results show red shifts in the Ex/Em parameters for Peaks A and C, increasing with increasing exposure times (15, 30, and 45 hours). Changes in the peak

bandwidth for Peak A were observed, with narrowing excitation width and broadening emission width at increasing exposure times. Exposed samples also show new fluorescence maxima, including a peak at 385/500. This peak may result from photochemical production of a new fluorophore, or possibly from biological production/reworking during exposure (we have not analyzed data on bacterial growth during exposure). However, a more likely explanation at this time is that this represents recalcitrant material that is masked in the original EEM, as the intensity overall decreases. Sample analysis should be complete by Winter 2002.

The second approach uses principal component analysis (PCA) for discrimination of small spectral differences between samples which in turn provide information of chemical composition of samples. Preliminary results from application of PCA to our EEM data demonstrate the potential of this technique to discern very subtle differences in spectra which could be used to an improved fingerprinting technique for organics in seawater. PCA was performed using Unscrambler software (Camo, ASA, Norway). CDOM concentration differences are first removed by mean-centering, thereby maximizing the spectral differences between samples.

PCA analyses showed changes in water masses across the Gulf of Mexico and within individual regions, with similar EEMs clustering together. PCA grouped samples from the mouth of Mississippi River water with samples taken from low salinity regions near Tampa Bay, in agreement with our hypothesis that this water mass can be attributed to the eastward migration of a Mississippi River plume. In addition, seasonal differences for coastal and offshore fluorescence endmembers in the Gulf of Mexico have been identified.

The technique has also been applied to a set of samples from ballast water exchange experiments conducted in collaboration with the Smithsonian Environmental Research Center. Transferable results from that analysis include demonstration of the usefulness of the technique in open ocean water samples from several major world ports, and discrimination of petroleum hydrocarbon contamination via PCA analysis.

We anticipate that this statistical data analysis tool will be useful for future application to EEMs generated using the MUVLIFS in real-time, underway mapping and profiling applications.

## **IMPACT/APPLICATIONS**

Development of a compact, *in situ*, UV multi-channel LIF sensor is at the cutting edge of scientific and technological development. Applications range from study of trace constituents in the atmosphere to tracking pollutants in natural waters. Miniaturization, multi-channel spectral capability, low detection limits and extension of detection into the UV are features now being rapidly advanced. As with most past examples of instrument development, the full range of applications and their value to science, defense, industry and society have yet to be fully explored.

## **TRANSITIONS**

Our work on this project is integrated with others focused on the West Florida Shelf study site (HYCODE), and provides critical background hydrographic data, such as CDOM and DOC distributions. Improved spectral discrimination of CDOM to elucidate its source and degree of biological and photochemical alteration will result in improved bio-optical models, which are an integral part of the HYCODE project objectives.

## RELATED PROJECTS

The MUVLIFS unit is targeted for multisensor applications to increase the level of certainty that a mine does or does not exist. We will test the performance of both shipboard and AUV systems in local waters in conjunction with an ongoing study jointly funded by the ONR-HYCODE Program and the EPA/NOAA EcoHAB Program. We will assess sensor performance for oceanographic applications.

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